EVALUATION OF SUPEROXIDE DISMUTASE LEVELS IN LOCAL DRUG DELIVERY SYSTEM CONTAINING 0.2% CURCUMIN STRIP AS AN ADJUNCT TO SCALING AND ROOT PLANING IN CHRONIC PERIODONTITIS: A CLINICAL AND BIOCHEMICAL STUDY

Dissertation submitted to

THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY

In partial fulfillment for the Degree of

MASTER OF DENTAL SURGERY

BRANCH II

PERIODONTICS

APRIL 2016

CERTIFICATE

This is to certify that **Dr.E.P.A.SANJAY ALEX,** Post Graduate student in the Department of Periodontics, J.K.K.Nattraja Dental College and Hospital, Komarapalyam has done this dissertation titled **"EVALUATION OF SUPEROXIDE DISMUTASE LEVELS IN LOCAL DRUG DELIVERY SYSTEM CONTAINING 0.2% CURCUMIN STRIP AS AN ADJUNCT TO SCALING AND ROOT PLANING IN CHRONIC PERIODONTITIS: A CLINICAL AND BIOCHEMICAL STUDY"** under my direct guidance during his post graduate study period 2013 - 2016.

This dissertation is submitted to **THE TAMILNADU Dr.MGR MEDICAL UNIVERSITY** in partial fulfillment of the degree of **MASTER OF DENTAL SURGERY, BRANCH II – Periodontics.**

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ACKNOWLEDGEMENT

*I am greatly indebted to **Dr.Sugumari Elavarasu,** my Professor and Head of the Department, Department of Periodontics, J.K.K.Nattraja Dental College and Hospital, Komarapalayam, for her strenuous and dedicated efforts towards the post graduate students and for her invaluable guidance, support and encouragement throughout my post graduate study.*

*I would like to extend my heartfelt gratitude to Professor **Dr.S.Sivakumar,** Principal, J.K.K.Nattraja Dental College and Hospital, for his kindness in allowing me to utilize the facilities in the college.*

*I am highly obliged to my sir **Dr.S.Thangakumaran,** Reader for his valuable suggestions, enthusiastic support and constant encouragement throughout my study.*

*I sincerely thank **Dr.T.Arthiie,** Reader for her valuable suggestions and generous support. Her profound knowledge, patience and perseverance and his incessant encouragement, guidance and support had benefited me and my colleagues in every facet of our academic life.*

*I take this opportunity to express my humble gratitude to **Dr. P.K. Sasi Kumar,** Reader, for his guidance and support throughout my study which had benefited my academic life.*

*My heartful thanks to all my **Postgraduate Colleagues,** and all the **Non-Teaching Staff's** for their kind help during my postgraduate period.*

I would like to thank all of my patients for their kind cooperation.

I also thank ***Dr. Ashish D. Wadhwani,*** *JSS College of Pharmacy, Ooty, for his constant and strenuous support in lab analysis.*

Words are not enough to express my love and affection to my father ***Mr. E.P.Alexander,*** *mother* ***Mrs. Kamala Alex*** *(Late), for their mental and physical support, without whom I would not have been able to reach this height.*

Finally, without the grace of the ALMIGHTY this possibility would have been just impossible.

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Periodontal disease is a common, chronic inflammatory condition that results in progressive destruction of tooth supporting tissues and formation of periodontal pockets between teeth and surrounding gingival tissues.[1] Periodontitis has a multi-factorial etiology, with primary etiological agents being pathogenic bacteria in the sub-gingival area. Among all the bacteria Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans are considered as an important periodontal pathogens causing destructive periodontal disease.[2]

The goals of periodontal therapy is not only to prevent, arrest, control or eliminate periodontitis, but also to restore the lost form, function, esthetics and comfort.[3] The standard periodontal therapy is with the objective of reducing the total bacterial load and changing the environmental conditions of the microbial niches. Although mechanical debridement (Scaling and Root planning) reduces the level of sub-gingival bacteria, but does not eliminate all the pathogens which reside deep into connective tissue which destroys the bone.[4]

To overcome the limitations of this conventional treatment, chemical agents such as antibiotics and antiseptics have been used successfully to treat moderate to severe periodontal diseases.[5] Systemic antibiotics require the administration of large doses to obtain suitable concentrations at the site of the disease which potentially promote the development of bacterial resistance, drug interactions, and inconsistent patient compliance.[6]

For the shortcomings of systemic administration, local delivery systems containing antibiotic or antiseptic agents were introduced.[7] These systems allow the therapeutic agents to be delivered directly to the diseased site with no appreciable

systemic effects. Various locally delivered agents that are successfully used include tetracycline fibres[8], 10% doxycycline[9,10], 2% minocycline[11], metronidazole[12] and chlorhexidine gluconate[13], but none are without side effects.

Research is being conducted for the use of the natural products instead of chemical agents. With the growing interest and increasing knowledge about the medicinal value of natural products, various formulations have been made commercially available.

Medicinal plants have been used as a traditional treatment agent for numerous human diseases since ages in many parts of the world. In rural areas of the developing countries, they continue to be used as the primary source of medicine. About 80% of the people in developing countries use traditional medicines for their health care. One such natural product which holds medicinal value is Turmeric (Curcuma longa).[14]

Curcumin has proven properties like anti-inflammatory, antioxidant, antimicrobial, hepatoprotective, immunostimulant, antiseptic, antimutagenic, and it also accelerates wound healing.[15]

Curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, Cm) is a multi-functional and pharmacologically safe natural agent. Curcumin is the principal curcuminoid of the popular Indian spice turmeric, which is a member of the ginger family (Zingiberaceae). The other two curcuminoids are desmethoxycurcumin and bis-desmethoxycurcumin. The curcuminoids are polyphones and are responsible for the yellow color of turmeric. Curcumin can exist in at least two tautomeric forms, keto and enol. The enol form is more energetically

stable in the solid phase and in a solution.[16] Turmeric *(haldi)* is a rhizome of *Curcuma longa* and may be a more acceptable and viable option for the common man.

Periodontal disease is widely believed to be initiated by microbial interaction, which triggers a host response by setting off an inflammatory reaction. Elevated proportions of some sub-gingival microbial species have been associated with destructive periodontal disease activity like Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythia, Peptostreptococcus micros, Campylobacter rectus, Eiknella corrodens, Fusobacterium nucleatum, Eubacterium species which leads to elevated levels of neutrophils.[17] Neutrophils are the key cells involved in the series of events, which includes release of inflammatory mediators and reactive oxygen species (ROS).

ROS include oxygen-derived free radicals, such as superoxide (O_2^-), hydroxyl (OH), nitric oxide, hydrogen peroxide, and hypochlorous acid (HOCl). Varieties of these molecules appear in the inflamed tissues and are capable of damaging lipids, proteins, and deoxyribonucleic acid, ultimately leading to tissue destruction. This oxidative stress phenomenon is believed in part to be responsible for the inflammatory conditions affecting the periodontium, manifesting as gingivitis and periodontitis.

Antioxidants are groups of substances that are able to prevent the oxidation of substrate by these ROS, thereby offering protection. Currently, there is a growing interest in the linkage between antioxidants and periodontal disease. A significant

antioxidant enzyme within mammalian tissues is superoxide dismutase, which catalyzes the dismutation of O_2^- to H_2O_2 and O_2. Superoxide dismutase has also been localized within the human periodontal ligament and may represent an important defense mechanism within gingival cells against superoxide release.[18]

The superoxide dismutase activity significantly improved following periodontal therapy, suggesting a positive response to nonsurgical periodontal therapy. Therefore, treatment of periodontal disease reduces oxidative stress by a concomitant reduction in inflammatory load by enhancing antioxidant levels, irrespective of the medium, GCF or saliva.[18] Curcumin is an Asian herbal medicine which offers antioxidant properties.[19]

The purpose of this study was to investigate the role of intrinsic antioxidants, superoxide dismutase enzyme, in the periodontal environment in GCF and the impact of nonsurgical treatment on the antioxidant profile using 0.2% Curcumin strip as local drug delivery.

The aim of the present study was

1. To evaluate the efficacy of 0.2% Curcumin (Curcuma Longa) strip as local drug delivery system in chronic periodontitis.

2. To compare the effectiveness of Curcumin strip with conventional periodontal mechanical therapy.

3. To investigate the role of intrinsic antioxidants, superoxide dismutase enzyme in response to Curcumin strip as local drug delivery.

Chronic periodontitis is an infectious disease resulting in inflammation within the supporting tissues of the teeth, progressive attachment loss, and bone loss and is characterized by periodontal pocket formation and/or recession of the gingiva.[20]

Elevated proportions of some sub-gingival microbial species have been associated with destructive periodontal disease activity like Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythia, Peptostreptococcus micros, Campylobacter rectus, Eiknella corrodens, Fusobacterium nucleatum, Eubacterium species.[21] The goal of periodontal therapy is to alter or get rid of the microbial etiology and to prevent, arrest, control or eliminate periodontitis and to restore the lost form, function, esthetics and comfort.[3]

Scaling and root planing is the most widely used approach that effectively decreases the microbial load.

NON SURGICAL PERIODONTAL THERAPY

Ramfjord et al. (1968)[22] compared the curettage and pocket elimination surgery in a longitudinal study for 2 years. He concluded that clinical attachment level was similar for both therapies.

Cercek et al. (1983)[23] conducted a study between two groups. One group comprises of initial therapy of oral hygiene instructions (OHI) and another group with scaling and root planing (SRP). According to him, OHI has minimal effects, while SRP groups had better PPD reduction and CAL gain.

Pihlstrom et al. (1983)[24] published the results of a study comparing SRP and SRP followed by Modified Widman flap (MWF) using a split mouth design, in 17 patients with moderate to advanced periodontal disease. Ten patients were available for examination at the conclusion of the study. The results showed that surgery led to CAL loss in 1-3mm sites, and both methods were equally effective in PPD reduction in 4-6mm sites, with SRP causing less CAL loss in the control site.

Cobb et al. (1996)[25] reported that significant reduction in probing depth and CAL at 3rd, 9th, 12th month following nonsurgical treatment of moderate to severe periodontitis.

Cugini et al. (2000)[26] reported that 70% reduction of probing depth in the mean number of pockets ≥ 4mm at 1 month and 4 months post treatment following non surgical treatment by manual scalers and curettes. He also reported that scaling and root planing resulted in significant decrease in DNA probe counts of a specific subset of sub- gingival microbes consisting of Porphyromonas gingivalis, Bacteroides forsythus and Treponema denticola.

Cobb CM (2002)[27] evaluated clinical significance of non-surgical periodontal therapy: an evidence based method. According to him, sub-gingival scaling effectively decreases the population of Gram negative microbes while concomitantly allowing for an increase in the populations of Gram positive rods and cocci.

Kursad S et al. (2002)[28] evaluated the effectiveness of Non-surgical periodontal treatment using 2 time intervals on short term healing and to assess patient reaction. The results revealed that significant decrease in plaque index,

gingival index, BOP, probing depth measurements at the end of third month, but no significant changes in gingival recession. The smallest time interval for non-surgical periodontal procedures might be 1 week.

Konopka et al. (2012)[29] studied the influence of scaling and root planing on amounts of IL-1β, IL-8 and MMP-8 in gingival crevicular fluid from patients with chronic periodontitis, in relation to clinical parameters. Observations indicated that short-term nonsurgical therapy resulted in a significant improvement in periodontal indices and a marked decrease of IL-1β, IL-8, MMP-8 gingival crevicular fluid levels.

In addition to mechanical treatment, the use of antimicrobial agents, both systemic and topical has been increasing because of the realization that periodontal disease is not merely an overgrowth of bacteria, but also shift in bacterial species.[30] Systemic administration of drug involves a relatively high dose with repeated intake over a prolonged period of time to achieve the required inhibitory concentrations in the sulcular fluid.[5]

Targeted drug delivery system is preferred over conventional drug delivery systems due to three main reasons:[31]

1. The first being pharmaceutical reason. Conventional drugs have low solubility and more drug instability in comparison to targeted drug delivery systems.

2. Conventional drugs also have poor absorption, shorter half-life and require large volume of distribution. These constitute its pharmacokinetic properties.

3. The third reason constitutes the pharmacodynamic properties of drugs. The conventional drugs have low specificity and low therapeutic index as compared to targeted drug delivery system. Due to these reasons targeted drug delivery system is preferred over conventional drug delivery systems.

Advantages of local drug delivery systems includes:[32]

i) Periodontal pockets are easily accessible and are therefore a convenient site for localized drug delivery systems which could be easily inserted into the pocket.

ii) It is useful in controlling and monitoring the desired drug levels in the site.

iii) It allows local modification of tissue permeability, inhibit protease activity or decrease immunogenic response.

iv) It is a useful means of delivering a drug to oral cavity that is not absorbed into he gastrointestinal system.

v) The drugs escape from the destructive acidic environment of the stomach.

LOCAL DRUG DELIVERY SYSTEMS

The local delivery of antimicrobial agents into the periodontal pocket is considered to have excellent potential as an adjunct to traditional periodontal therapy. Delivery of therapeutic levels of antibacterial agents directly into the pocket with no systemic side effects, however the success of this treatment is the ability to control and to prolong the release rate of the therapeutic agent from the device.[33]

MOUTH RINSES/DENTRIFICES:

Tinanoff et al. (1980)[34] evaluated the effect of stannous fluoride mouth rinse on dental plaque formation. They concluded that mouthrinses and dentrifices are inefficient because of the short period of contact of the drug with the tissues and the lack of penetration into the periodontal pocket.

FIBRES:

Addy et al. (1982)[35] showed that chlorhexidine was released from reservoir fibres over 4 days in vitro and more than 95% of the drug release occurred in the first 24 hour. As the drug was placed at effective levels for 24 hours, placement of single tubing does not provide sufficient treatment to prevent pocket recolonization.

INJECTABLE DEVICES:

OINTMENT:

Van Steenberghe D et al. (1994)[36] evaluated the effect of 2% minocycline ointment as an adjunct to scaling and root planing. They concluded that ointment does not appear to have any sustained release properties.

GEL FORM:

Unsal et al (1994)[37] reported that chlorhexidine gel results in reduction in probing pocket depth, which are not significantly different from the result obtained from scaling and root planing. Single application of gel is not sufficient to produce reduction in clinical parameters.

FILMS:

Films are matrix delivery systems in which the drug is distributed throughout the polymer and release occurs by drug diffuse or matrix dissolution or erosion.

Soskoline et al. (1996)[38] reviewed the intra periodontal pocket drug delivery systems. They concluded that films has several advantageous physical properties such as dimensions and shape of the film can be easily controlled, correspond to the dimension of the pocket, minimal discomfort to the patients, it will remain submerged without noticeable interference to the patient's oral hygiene habits.

Vyas SP, Sihorkar and Mishra (2000)[39] reviewed the research paper on controlled and targeted drug delivery strategies towards intra-periodontal pocket diseases. Several degradable and non-degradable devices are under investigation for the delivery of antimicrobial agents into the periodontal pocket including non-biodegradable fibres, films (biodegradable and non-biodegradable), bio-absorbable dental materials, biodegradable gels/ ointments, injectables and microcapsules. Similarly, bioadhesive delivery systems are explored that could significantly improve oral therapeutics for periodontal disease and mucosal lesions.

Arthur J et al. (2005)[40] reviewed impact of local adjuncts to scaling and root planing in periodontal disease therapy. The results showed that among the locally administered adjunctive antimicrobials, positive results occurred for tetracycline, minocycline, metronidazole, and chlorhexidine. Adjunctive local therapy generally reduced the probing depth levels.

Greenstein G. (2006)[41] reviewed clinical significance of local drug delivery in the treatment of periodontal diseases. According to him, local drug delivery

devices when used as adjuncts to SRP provided a statistically significant enhancement of parameters commonly used to monitor periodontal status.

Pragati et al. (2009)[42] published the results of Pinon et al study. According to them, in vivo study in dogs with induced periodontal defects using Triclosan-loaded polymeric nanoparticles and suggested that triclosan loaded nanoparticles penetrate through the junctional epithelium. Dung et al used antisense oligonucleotide- loaded chitsantripolyphosphate (TPP) nanoparticles and showed the sustained release of oligonucleotides which is suitable for the local therapeutic application in periodontal diseases.

LOCAL DRUG DELIVERY AGENTS:

CHLORHEXIDINE:

Kinane DF and Radvar M.et al (1999)[43] – 6 months follow-up parallel study was to evaluate the efficacy of three commercially available local delivery system as adjuncts to scaling and root planing in the treatment of sites with persistent periodontal lesions. Scaling and root planing alone (SRP) (20 patients), or in conjunction with the application of 25% tetracycline fibers (S+Tet) (19 patients), or 2% minocycline gel (S+Min) (21 patients), or 25% metronidazole gel (S+ Met) (19 patients). The results showed that 3 locally applied antimicrobial systems seem to offer some benefit over SRP alone, a treatment regimen of scaling and root planing plus tetracycline fiber placement gave the greatest reduction in probing depth over the 6 months after treatment.

Grisi et al. (2002)[44] evaluated the effectiveness of a controlled released chlorhexidine chip (CHX) as adjunctive therapy to scaling and root planing (SRP) in

the treatment of chronic periodontitis. The CHX chip did not provide any clinical or microbiological benefit beyond that achieved with conventional scaling and root planing, after a 9 months period.

Jan Cosyn et al. (2006)[45] reviewed the effects of the chlorhexidine chip when used as an adjunct to scaling and root planing in the treatment of chronic periodontitis. Multicenter studies have indicated significantly higher pocket reductions and clinical attachment gains following a combination of mechanical debridement and repeated planing alone.

DOXYCYCLINE:

Martorelli de Lima et al (2004)[46] evaluated the effect of subgingival administration of doxycycline as an adjunct to periodontal therapy in type 1 diabetes mellitus (DM) patients. The findings suggested that subgingivally delivered doxycycline hyclate produces additional favorable clinical results to periodontal therapy in type 1 DM patients.

Machion L et al (2008)[47] evaluated the efficacy of locally delivered doxycycline (10%) with scaling and root planing in the treatment of periodontal diseases in smokers. Relative attachment level gain was greater for SRP-D than SRP alone. They concluded that locally delivered doxycycline may constitute an important adjunct in the treatment of severe periodontal diseases.

Maurizio S, Tonetti, et al. (2012)[48] evaluated the efficacy of a slow release doxycycline gel (SRD) adjunctively administered to non-surgical therapy in subjects with recurrent or persistent periodontitis. The trial results showed that topically administered SRD may provide short-term benefit in controlling inflammation and

deep pockets in treated periodontal patients participating in a secondary prevention programme and able to maintain a satisfactory level of oral hygiene.

TETRACYCLINE:

Lynn R et al. (2002)[49] evaluated the efficacy of tetracycline strips administered single or in multiples in conjunction with root planning, versus root planing alone, or to an untreated control. The data suggested that multiple strips are superior to single strips in reducing bleeding on probing and that local delivery of tetracycline is superior to root planning alone in reducing probing depth.

Aimetti et al. (2004)[50] evaluated the clinical, radiological and microbiological response to the local delivery of tetracycline in sites with persistent periodontal lesion. SRP plus tetracycline fibers gave the greatest advantage in the treatment of periodontal persistent lesions atleast 12 months following treatment.

METRONIDAZOLE:

Greenstein et al. (1998)[51] suggested that there was a better result over a 9-month observation period when combined therapy (metronidazole gel plus scaling and root planing) was employed for probing depth reduction.

Shifrovitch Y et al. (2009)[52] evaluated Metronidazole – loaded bioabsorbable films as local antibacterial treatment of infected periodontal pockets. The results showed that the developed systems demonstrate good biocompatibility, therefore may be useful in the treatment of periodontal diseases.

MINOCYCLINE:

Van Steenberghe et al. (1999)[53] evaluated the long term safety and efficacy of sub-gingivally administered minocycline ointment versus a vehicle control. Overall results demonstrated that repeated sub-gingival administration of minocycline ointment in the treatment of adult periodontitis is safe and leads to significant adjunct improvement after sub-gingival instrumentation in both clinical and microbiological variables over a 15 months period.

CLARITHROMYCIN GEL

Agarwal et al. (2012)[54] conducted a study to investigate the adjunctive effects of subgingivally delivered 0.5% clarithromycin as an adjunct to scaling and root planing for treating chronic periodontitis smoker subjects. It was observed that the adjunctive use of 0.5% clarithromycin as a controlled drug delivery system enhanced the clinical outcomes. At the end of 6 months, the mean GI, PI, SBI, PPD, CAL for the clarithromycin group were significantly reduced.

OFLOXACIN

Nakahara et al. (2003)[55] reviewed in his article as Okade and co-workers in 1999 developed a new subgingival release delivery system containing Ofloxacin for sub gingival therapy. It was found to be effective in the reduction of supra gingival plaque, reduction in plaque index, reduction in bleeding on probing.

HERBS IN LOCAL DRUG DELIVERY AGENTS

FOLATE MOUTHWASH:

Pack ARC. (1984)[56] evaluated the effect of folate mouth wash on established gingivitis. He concluded that the patients using folate mouth washes had reduced bleeding, plaque accumulation when compared to controls.

EUCALYPTUS EXTRACT:

Nagata et al. (2008)[57] demonstrated that the subjects who chewed eucalyptus containing gum found relief from the symptoms including less gingival bleeding, reduced pocket depth and reduced plaque accumulation. Using gum, toothpaste or tinctures containing eucalyptus extract could benefit to gingiva and mouth.

SANGUINARINE:

Vogel et al. (1978)[58] studied that due to its natural alkaloids, bloodroot can curb the growth of bacteria responsible for gum disease. Sometimes included in oral health products such as toothpaste and mouthwashes, this herb can calm inflammation and prevent bacteria from deepening of periodontal pockets, which helps to halt the bone decay that eventually leads to tooth loss.

Tenenbaum et al. (1999)[59] in a 14 week controlled clinical trial supported the combined use of chlorhexidine mouthwash for 2 weeks followed by sanguinaria mouth rinse and tooth paste upto 3 months in treating periodontitis.

CHAMOMILE:

Chopra et al. (1956)[60] reviewed the anti-inflammatory and antibacterial properties of chamomile that can help to soothe inflammation from periodontitis and reduce the levels of unhealthy bacteria in the mouth.

Kopiga Ananthathavam. (2014)[61] Chamomile helps to reduce inflammation from periodontitis and also reduces the level of unhealthy bacteria in the mouth. In order to expose the gum to this herb, Chamomile tea is taken or mouthrinses and toothpastes containing Chamomile is taken to overcome periodontal infections.

GREEN TEA CATECHIN:

Praveen Kudwa et al. (2010)[62] evaluated the adjunctive use of locally delivered Green tea catechin with scaling and root planing as compared to SRP alone in the treatment of chronic periodontitis. The results revealed that significant probing depth reduction in the test group when compared to controls along with microbiological parameters.

Jayaprakash S Gadagi et al. (2013)[63] conducted a study and incorporated green tea extract into hydroxylpropyl methylcellulose and investigates its efficacy in chronic periodontitis patients associated with and without diabetes mellitus. Results showed that Both groups showed significant reduction in GI scores , PPD reduction and CAL gain at the test sites.

ALOE VERA:

Harjit Kaur Virdi et al. (2012)[64] evaluated the effect of locally delivered aloe vera gel as an adjunct to scaling and root planing in the treatment of chronic

periodontitis. They concluded that SRP-Aloe vera group showed significantly better results than SRP alone in 6 weeks.

TULSI (Ocimum sanctum):

Sen P. (1993)[65] reviewed the therapeutic potential of tulsi. According to him, Tulsi plant had shown to possess anti-stress, anti-hyperlipidemic, analgesic and anti-microbial properties. The anti-bacterial property was utilized, Tulsi as an effective drug in the control of oral pathogenic bacteria causing diseases.

ACACIA CATECHU WILD:

Ray et al. (2006)[66] reviewed the antipyretic, antidiarrhoeal, hypoglucemic and hepato-protective activities of ethyl acetate extract of Acacia catechu in albino rats. A. catechu is used as mouthwash for mouth, gum and throat disease like gingivitis and stomatitis.

NEEM (Azadirachta indica):

Sanjeev Jain et al. (2012)[67] evaluated the efficacy of Neem chip as an adjunct to scaling and root planing in patients with periodontitis. The results showed that significant reduction in probing depth in Neem chip group when compared to SRP alone group.

TURMERIC (Curcuma longa):

The origin of the plant *Curcuma longa L*. (Family *Zingiberaceae*) is India. The plant is distributed throughout tropical and subtropical regions of the world, being widely cultivated in southeast as a spice, mainly as an ingredient in many

varieties of curry powder and sauces, where *curcumin* from turmeric is a main colouring substances. The *rhizome* of turmeric has been used in Asian cookery, medicine, cosmetics and fabric dying for more than 2000 years. Early European explorers to the Asian continent introduced this important spice to the Western world in 14th century.[68]

Various names of turmeric in different languages:[16]

Language	Name
Arabic	Kurkum, Uqdah safra
Bulgarian	Kurkuma
Chinese	Yu chin, Yu jin, Wohng geung, Geung wohng, Wat gam, Huang jiang, Jiang huang, Yu jin, Yu jin xiang gen
English	Indian saffron
French	Curcuma, Safran des Indes, Terre-mérite, Souchet des Indes
German	Curcuma, Kurkuma, Indischer Safran, Gelbwurz
Hindi	Haldi
Japanese	Ukon, Tamerikku
Russian	Koren, kurkumy, Kurkuma
Sanskrit	Ameshta, bahula, bhadra, dhirgharaja,
Tamil	Manjal
Ukrainian	Kurkuma
Vietnamese	Bot nghe, Cu nghe, Nghe, Uat kim, Khuong hoang
Yiddish	Kurkume.

Thakur R (1989)[69] In Northern India, women are given a tonic of fresh turmeric paste with powder of dried ginger roots and honey in a glass of hot milk to drink twice daily after childbirth. Apoultice of turmeric is also applied to the perineum to aid in the healing of any lacerations in the birth canal. Powdered turmeric is taken with boiled milk to cure cough and related respiratory ailments, and roasted turmeric is an ingredient used as an antidysenteric for children.

The benefits of curcumin include analgesic, antimicrobial, antioxidant, astringent, antispasmodic, appetizer, carminative and in the recent years have been found to be quite useful in dentistry. Biological activities are an antioxidant, anti-inflammatory agent, an immunomodulator Anti tumorogenic healing agent and an antimicrobial agent.[70]

Bhandari and Shankwalkar. (1980)[15] used turmeric in the form of a mouthwash. Similar reductions have been attributed to the use of turmeric in pure and water-soluble form as a consequence of its anti-inflammatory property.

Singh R. (2002)[71] shown that curcumin significantly inhibited *P.gingivalis* LPS (Lipopolysaccharide) induced TNFα and IL-1β production and the inhibition of these cytokines by curcumin may contribute to reducing the impact of cytokine-mediated tissue destructive process in periodontitis.

Gopinath et al. (2004)[71] showed that curcumin incorporated in collagen, which acts as supportive matrix for slow release, increases wound reduction and enhances cellular proliferation.

Suhag et al. (2007)[72] in his study treated periodontal sites by SRP. Selected sites were irrigated with either saline (0.9%), chlorhexidine (0.2%), curcumin (1%),

or served as nonirrigated control sites on day 0 (baseline) immediately following instrumentation. Triple irrigation regimen was repeated for the next 5 consecutive days and on days 15 and 21. The results indicated that the irrigated sites had significant improvement in all parameters as compared with the nonirrigated sites on days 2, 3, 4, and 5. The curcumin group showed significant reduction in BOP (100%) and redness (96%) when compared with the chlorhexidine group and saline group on day 5.

Kim SJ. (2011)[68] states that curcumin can inhibit *P.gingivalis* LPS -induced cytokine expression. According to the author, Curcumin strongly suppressed the production of IL-6 at both gene transcription and translation levels in *Prevotella intermedia* LPS-activated RAW 264.7 cells.

In a study by **Waghmare et al. (2011)[14]** about 100 subjects were randomly selected. Both gingival index and plaque index were recorded at 0, 14, and 21 days. It was concluded that chlorhexidine gluconate as well as turmeric mouthwash can be effectively used as an adjunct to mechanical plaque control methods in prevention of plaque and gingivitis.

Behal et al. (2011)[15] conducted a study with 30 subjects with chronic localized or generalized periodontitis with pocket depth of 5‑7 mm were enrolled in a split‑mouth study design. Control sites received Scaling and Root Planing [SRP] alone, while experimental sites received SRP plus 2% whole turmeric gel. Both groups demonstrated statistically significant reduction in plaque index, gingival index, sulcus bleeding index, probing pocket depth, and gain in relative attachment

loss. There was a significant reduction in the trypsin‑like enzyme activity of "red complex"microorganisms.

Nayyar Nandini et al. (2012)[73] evaluated the clinical and microbiological efficacy of subgingival irrigation with curcumin post scaling and root planing when compared to the gold standard chlorhexidine. Result shows chlorhexidine irrigation appeared to provide greater plaque and gingivitis inhibitory action which was followed by curcumin irrigation was slightly lower than chlorhexidine and then 0.9% saline irrigation which was less effective and significantly lower when compared to curcumin and chlorhexidine irrigation.

Sruthima N et al. (2014)[74] comparatively evaluate the therapeutic efficacy of chlorhexidine (CHX) chips (Periocol-CG) and indigenous curcumin (CU) based collagen as adjuncts to scaling and root planning in the nonsurgical management of chronic periodontitis. The results showed that significant reduction in plaque and gingival index scores were observed in both groups at the end of the study period, i.e., 6 months. The microbiological parameters (BANA test, microbial colony count), PPD and CAL levels also showed significant improvement in both groups. However, at the end of the study period CHX group showed greater improvement in all of these parameters compared to CU collagen group.

Madhu Bhatia et al. (2014)[69] evaluate the clinical and microbiological efficacy of locally delivered 1% curcumin gel as an adjunct to scaling and root planing in the treatment of chronic periodontitis. The study results appeared to provide significant improvements in clinical parameters. Microbiological counts of Porphyromonas gingivalis, Prevotella intermedia, Fusobacterium nucleatum and

capnocytophaga showed significant reduction in periopathogens at the test sites after six months when compared with that of control sites.

Merline K Varghese (2014)[75] compared the clinical efficacy between two medicaments, delivered in gel form, one containing metronidazole and other containing curcumin, as an adjunct to mechanotherapy. The results showed that when compared with metronidazole, a slight decrease in all clinical parameters was seen in the curcumin group. The study revealed that the experimental local drug i.e. curcumin used along with SRP is effective in reducing gingival inflammation and PPD.

A randomized, split mouth controlled clinical study was conducted to evaluate the effectiveness of Curcumin strip in the treatment of chronic periodontitis compared to SRP alone. The protocol was reviewed and approved by institutional ethical board. The study related procedures were explained to the patients before they sign an informed consent form. A total of 15 subjects each with bilateral 5-6 mm probing pocket depth (PPD) were recruited from the outpatient Department of Periodontics, J. K. K. Nattraja Dental College and Hospitals, Komarapalayam, Tamilnadu based on the following criteria.

Inclusion criteria:

1. Age limit of 35-55 years of both sexes.
2. Probing pocket depth (PPD) of 5-6 mm bilaterally as assessed by Williams periodontal probe.
3. Vital teeth.
4. Systemically healthy patients.
5. Ability to maintain good oral hygiene.

Exclusion criteria:

1. Patients received treatment with any form of antibiotics/ anti- inflammatory drugs in last 3 months.
2. Smokers.
3. Patients with history of systemic diseases.
4. Pregnant and lactating women.
5. History of drug allergies.

STUDY DESIGN:

A split mouth design was followed, where two sites with probing pocket depth of 5-6 mm were chosen. Probing pocket depth standardization was done with acrylic stent in all the selected areas.

CRITERIA FOR GROUPING

Selected sites were randomly divided into control sites and experimental sites as follows:

- Healthy group – It consists of 5 sites in which no bleeding on probing , no pocket depth. (Healthy group).
- Group I – It consists of 15 sites, in which scaling and root planning (SRP) was done (control sites).
- Group II – It consists of 15 sites, in which scaling and root planning was followed by the placement of Curcumin strip inside the pocket (SRP + Curcumin strip) (Test sites).

CLINICAL PARAMETERS

The following variables were measured at baseline and at 21 days

1. Plaque index (PI) (Silness and Loe 1964)
2. Gingival index (GI) (Loe and Silness 1963)
3. Sulcus bleeding index (SBI) (Muhlemann and Son 1971)
4. Probing pocket depth (PPD)

1. Plaque index: (Silness and Loe 1964)

The four gingival areas of the tooth surfaces examined are the disto-facial, facial, mesio-facial and lingual surfaces.

Scoring was as follows:

0 – No plaque.

1 – A film of plaque adhering to the free gingival margin and adjacent area of the tooth. The plaque may be seen only by running a probe across the tooth surface.

2 – Moderate accumulation of soft deposits within the gingival pocket, on the gingival margin and/or adjacent tooth surface, which can be seen by the naked eye.

3 – Abundance of soft matter within the gingival pocket and/or on the gingival margin and adjacent tooth surface.

The scores of the four areas of the tooth can be summed and divided by four to give the PI for the tooth. A score from

0.1 – 0.9 – Good.

1.0 – 1.9 – Fair.

2.0 – 3.0 – Poor.

2. Gingival index: (Loe and Silness 1963)

The gingival index was created for the assessment of the gingival condition and records qualitative changes in the gingival. It scores the marginal and interproximal tissues separately on the basis of 0 to 3. The criteria are:

0 – Normal gingiva.

1 – Mild inflammation – slight change in color and slight edema but no bleeding on probing.

2 – Moderate inflammation – redness, edema and glazing, bleeding on probing.

3 – Severe inflammation – marked redness and edema, ulceration with tendency to spontaneous bleeding.

The scores of the four areas of the tooth can be summed and divided by four to give the GI for the tooth. A score from

0.1 – 1.0 – Mild inflammation.

1.1 – 2.0 – Moderate inflammation.

2.1 – 3.0 – Signifies severe inflammation.

3. Sulcus bleeding index: (Muhlemann and Son, 1971)

The criteria for scoring are as follows:

0 – Healthy looking papillary and marginal gingiva, no bleeding on probing.

1 – Healthy looking gingival, bleeding on probing.

2 – Bleeding on probing, change in color, no edema.

3 - Bleeding on probing, change in color, slight edema.

4 - Bleeding on probing, change in color, obvious edema.

5 – Spontaneous bleeding, change in color, marked edema.

Four gingival units are scored systematically for each tooth: the labial and lingual marginal gingiva (M units) and the mesial and distal papillary gingiva (P units). Scores for these units are added and divided by four. Adding the scores of the undivided teeth and dividing them by the number of teeth can determine the sulcus bleeding index.

4. Probing pocket depth (PPD) was recorded was measured at selected sites using William's graduated periodontal probe. The probe was inserted parallel to the long axis of the tooth gently, until resistance was felt and the readings were recorded to the nearest millimeter from the gingival margin to the base of the pocket. Acrylic stents were used to standardize the path of insertion and angulations of the probe.

MTT (3-(4,5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) assay:

Cell viability was assessed by MTT (3-(4,5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) method.

Principle

Tetrazolium salts from non-water soluble formazan crystals within the cells, to form a colored complex in addition of solubilising solution, which can read at 650nm in spectrometer.

Reagents

1. MTT (0.5%):

   0.25g MTT was dissolved in 50ml of serum free RPMI medium.

2. Solubilizing solution:

   (20% Sodium Lauryl Sulfate (SDS) in 50% dimethylformamide (DMF)

   5.0ml DMF was made up to 10 ml with distilled water and 2g of SDS were added and mixed well.

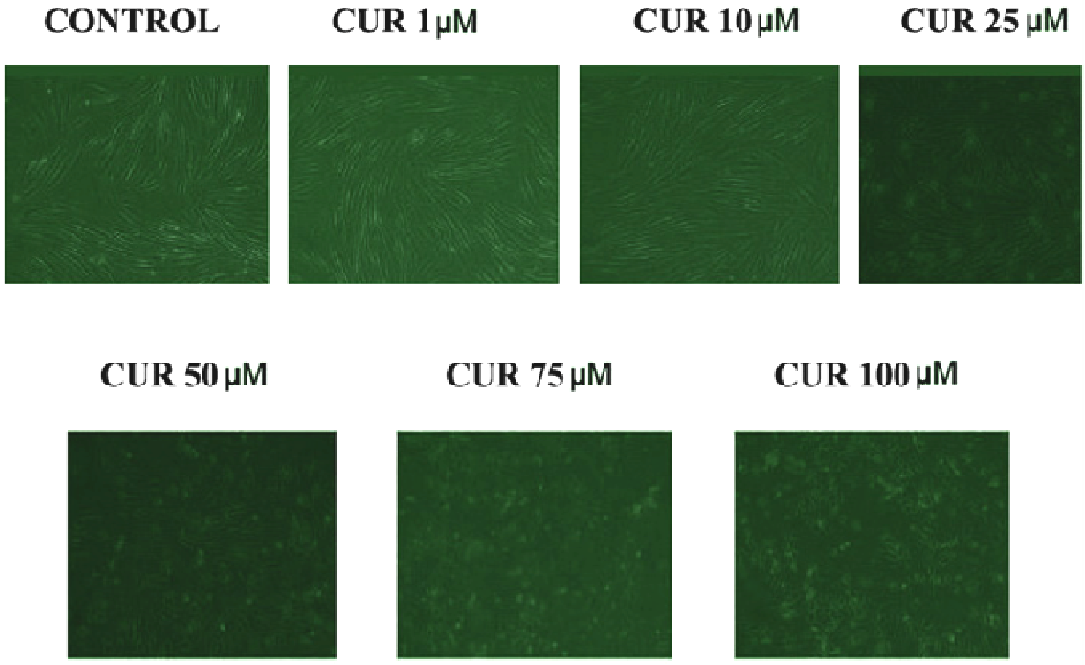
Experimental protocol for MTT assay:

Fibroblast cells were placed on 24 well plates on the surface of membrane at a concentration of 5×10^4 cells/ml and incubated at 37º C in a humidified atmosphere of 5% CO_2.

After 24 hrs of culture, the medium was removed. In the cultured plate 100 µl of 0.5% MTT was added and then incubated at 37ºC for another 4 hour. After

incubation, the MTT containing medium was removed from the plate and 100µl of solubilizing solution was added to each well to dissolve the formazan crystals. The cell viability calculated as percentage of viable cells and then plotted on a graph.

Metabolic activity of mitochondria was measured using spectrophotometer (Molecular Devices, Sunnyvale, USA). The optical density (OD) was determined at a wavelength of 690nm. The experiments were repeated three times with tetrad samples.



HIGH PERFORMANCE LIQUID CHROMATOGRAPHY(HPLC) ANALYSIS METHOD:

All the samples were analyzed by the WATERS® HPLC system using Perkin Elmer HPLC column (SPHERI–5 RP – 18, Perkin Elmer LLC, Norwalk city, CT, USA) with the following specifications 5µm, 4.6mm×250mm Preceded by a guard column (SecurityGurad™, Phenomenex Inc., Torrance, CA, USA) filled with C18 cartridges (4 x 2.00 mm ID) at room temperature. The mobile phase was

Methanol: H2O (containing 3.6% glacial acetic acid) (73:27, v/v), freshly prepared on the day of use, filtered through a 0.45 µm filter and was degassed by sonication for 15 min **(Dandekar and Patravale, 2009)**. Curcumin was detected at 428 nm with a sample run time of 10 min and a flow rate of 1ml/min.

In-Vitro Release Profile of curcumin:

The cumulative drug released percent (CDR %) was determined as described earlier with a suitable modification (**Nayak et al., 2010; Bisht et al., 2007**). Known amount of the collagen impregnated curcumin (100 mg) were dispersed in PBS pH 7.4 and the dispersion was divided into 8 aliquots (1 ml each) in microfuge tubes which were kept in a thermo stable water bath at 37°C. At predetermined time intervals, the dispersion was centrifuged at 200×g for 5min and the resulted pellets were then re-dissolved in 1ml of methanol. After that, the drug content was determined using HPLC analysis method and the concentration of the released curcumin was calculated using a standard curve of curcumin in methanol.

Release Kinetics of curcumin from Collagen membrane:

The kinetic parameters for the in-vitro release of curcumin from the developed collagen membrane were determined and then analyzed in order to find the proper order of the drug release using PCP Disso software v3.00 (Pune, India).

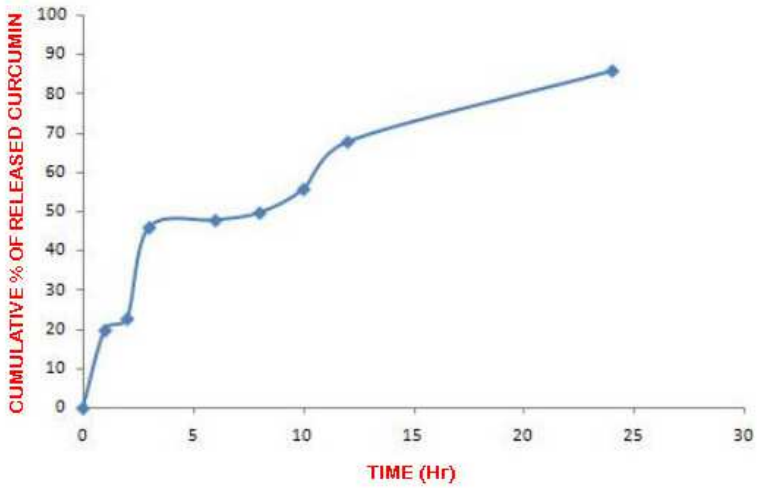
RESULT OF HPLC analysis method:

The employed HPLC analysis method gave a sharp peak of curcumin, without tailing, with a retention time of around 6.3 min at 428 nm.

In-Vitro Release Profile of curcumin and the Kinetic treatment figure represented the release profile of curcumin from the developed collagen membrane formula over a period of 24 hours. The figure shows the cumulative percentage release of curcumin very clearly in figure.

GRAPH – 1

The in-vitro release of curcumin from the Collagen membrane over 24 hr



ENZYME LINKED IMMUNOSORBENT ASSAY:

The kit is a sandwich enzyme immunoassay for in vitro quantitative measurement of superoxide dismutase in human serum, plasma, tissue homogenates, cell lysates and other biological fluids. An anti-human SOD coating antibody is adsorbed onto microwells. Human SOD present in the sample or standard binds to antibodies adsorbed to the microwells. A HRP-conjugated anti-human SOD antibody is added and binds to human SOD captured by the first antibody. Following incubation unbound HRP- conjugated anti-human SOD is removed

during a wash step, and substrate solution reactive with HRP is added to the wells. A coloured product is formed in proportion to the amount of human SOD present in the sample or standard. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from 7 human SOD standard dilutions and human SOD concentration determined.

REAGENTS PROVIDED WITH KIT:

Reagents for human SOD ELISA ALX-850-033

a) 1 Aluminium pouch with a Microwell Plate coated with monoclonal antibody to human SOD.

b) 1 Vials (20 µl) HRP-Conjugate anti-human SOD monoclonal antibody

c) 2 vials (500 µl) human SOD Standard, 5 ng/ml

d) 1 vial (5 ml) Assay Buffer Concentrate 20x (PBS with 1% Tween 20 and 10% BSA)

e) 1 vial (5 ml) Phosphate Buffered Saline Concentrate (PBS) 20x

f) 1 bottle (50 ml) Wash Buffer Concentrate 20x (PBS with 1% Tween 20)

g) 1 vial (15 ml) Substrate Solution (tetramethyl - benzidine)

h) 1 vial (15 ml) Stop Solution (1M Phosphoric acid)

i) 1 vial (0.4 ml) Blue-Dye

j) 1 vial (0.4 ml) Green-Dye

k) 2 Adhesive Films

MATERIALS REQUIRED BUT NOT AVAILABLE WITH THE KIT:

1. 5 ml and 10 ml graduated pipettes
2. 5 µl to 1000 µl adjustable single channel micropipettes with disposable tips
3. 50 µl to 300 µl adjustable multichannel micropipette with disposable tips
4. Multichannel micropipette reservoir
5. Beakers, flasks, cylinders necessary for preparation of reagents
6. Device for delivery of wash solution (multichannel wash bottle or automatic wash system)
7. Microwell strip reader capable of reading at 450 nm (620 nm as optional reference wave length)
8. Glass-distilled or deionized water
9. Statistical calculator with program to perform regression analysis

PREPARATION OF REAGENTS

Buffer Concentrates should be brought to room temperature and should be diluted before starting the test procedure. If crystals have formed in the Buffer Concentrates, warm them gently until they have completely dissolved.

Wash Buffer (1x):

Pour entire contents (50 ml) of the Wash Buffer Concentrate (20x) into a clean 1000 ml graduated cylinder. Bring to final volume of 1000 ml with glass-distilled or deionized water.

Mix gently to avoid foaming.

Transfer to a clean wash bottle and store at 2° to 25°C.

Please note that Wash Buffer (1x) is stable for 30 days.

Phosphate Buffered Saline (PBS) (1x):

Mix the contents of the bottle well. Add contents of PBS concentrate (5.0 ml) to 95 ml distilled or deionized water and mix gently to avoid foaming. Store at 2° to 8°C. Please note that the PBS (1x) is stable for 30 days.

HRP-Conjugate:

Please note that the HRP-Conjugate should be used within 30 minutes after dilution. Dilute the HRP-Conjugate 1:5 just prior to use by adding 80 µl Assay Buffer (1x) to the tube containing the HRP Conjugate concentrate. Mix the contents of the tube well.

Human SOD Standard:

Standard dilutions can be prepared directly on the microwell plate or alternatively in tubes.

External Standard Dilution:

Label 7 tubes, one for each standard point. S1, S2, S3, S4, S5, S6, S7 Then prepare 1:2 serial dilutions for the standard curve as follows:

Pipette 225 µl of PBS (1x) into tubes S2 – S7.

Pipette 450 µl of undiluted standard (serves as the highest standard S1, concentration of standard 1= 5 ng/ml) into the first tube, labelled S1.

Pipette 225 µl of this dilution into the second tube, labelled S2 (concentration of standard 2 = 2.5 ng/ml), and mix thoroughly before the next transfer.

Repeat serial dilutions 5 more times thus creating the points of the standard curve

Dye:

The dye solutions from the stocks provided (*Blue-Dye*, *Green-Dye*) can be added to the reagents according to the following guidelines:

Diluent:

Before standard and sample dilution add the Blue-Dye at a dilution of 1:250 to the appropriate diluent (1x) according to the test protocol. After addition of Blue-Dye, proceed according to the instruction booklet.

HRP-Conjugate:

Before dilution of the concentrated HRP-Conjugate add the Green Dye at a dilution of 1:100 to the Assay Buffer (1x) used for the final conjugate dilution. Proceed after addition of Green-Dye according to the instruction booklet: Preparation of HRP-Conjugate.

ASSAY PROCEDURE:

Determine the number of microwell strips required to test the desired number of samples plus appropriate number of wells needed for running blanks and standards. Each sample, standard, blank and optional control sample should be assayed in duplicate. Remove extra microwell strips from holder and store in foil bag with the desiccant provided at 2°-8°C sealed tightly.

Wash the microwell strips twice with approximately 400 µl WashBuffer per well with thorough aspiration of microwell contents between washes. Allow the Wash Buffer to sit in the wells for about 10 – 15 seconds before aspiration. Take care not to scratch the surface of the microwells. After the last wash step, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffer. Use the microwell strips immediately after washing. Alternatively microwell strips can be placed upside down on a wet absorbent paper for not longer than 15 minutes. Do not allow wells to dry.

Add 100 µl of PBS (1x) in duplicate to standard wells B1/2- G1/2, leaving A1/A2 empty. Pipette 200 µl of undiluted standard (concentration = 5.00 ng/ml) in duplicate into well A1 and A2. Transfer 100 µl to wells B1 and B2. Mix the contents of wells B1 and B2 by repeated aspiration and ejection, and transfer 100 µl to wells

C1 and C2, respectively. Take care not to scratch the inner surface of the microwells. Continue this procedure 4 times, creating two rows of humanSOD standard dilutions ranging from 5.00 to 0.08 ng/ml. Discard 100 µl of the contents from the last.

Add 100 µl of PBS (1x)in duplicate to the blank wells.

Add 90 µl of PBS (1x)to the sample wells.

Add 10 µl of each prediluted sample in duplicate to the sample wells.

Prepare HRP-Conjugate.

Add 50 µl of HRP-Conjugate to all wells.

Cover with an adhesive film and incubate at room temperature (18 to 25°C) for 1 hour, if available on a microplate shaker set at 100 rpm.

Remove adhesive film and empty wells. Wash microwell strips 3 times according to point 0. of the test protocol. Proceed immediately to the next step.

Pipette 100 µl of TMB Substrate Solution to all wells.

Incubate the microwell strips at room temperature (18° to 25°C) for about 10 min. Avoid direct exposure to intense light.

The colour development on the plate should be monitored and the substrate reaction stopped before positive wells are no longer properly recordable. Determination of the ideal time period for colour development has to be done individually for each assay.

It is recommended to add the stop solution when the highest standard has developed a dark blue colour. Alternatively the colour development can be monitored by the ELISA reader at 620 nm. The substrate reaction should be stopped as soon as Standard 1 has reached an OD of 0.9 – 0.95.

Stop the enzyme reaction by quickly pipetting 100 µl of Stop Solution into each well. It is important that the Stop Solution is spread quickly and uniformly throughout the microwells to completely inactivate the enzyme. Results must be read immediately after the Stop Solution is added or within one hour if the microwell strips are stored at 2-8°c in the dark.

Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length (optionally 620 nm as the reference wave length; 610 nm to 650 nm is acceptable). Blank the plate reader according to the manufacturer's instructions by using the blank wells. Determine the absorbance of both the samples and the standards.

APPENDIX-1

POST THERAPY INSTRUCTIONS

- Report immediately on development of any untoward reactions like pain, swelling, bleeding and drug allergies.
- Should avoid intake of any hard and hot foods, not to disturb the operated area with tongue.
- Report if dressing is dislodged.
- Avoid brushing the treated area from the day of therapy. Use cotton applicator to gently clean the area and resume gentle brushing with soft brush and coronally directed roll technique.
- Avoid the use of medications and mouthrinses.
- To report as per schedule.

PROFORMA

NAME: DATE:

AGE: SEX: OP NO:

OCCUPATION:

ADDRESS:

CHIEF COMPLAINTS:

DENTAL HISTORY:

MEDICAL HISTORY:

PERSONAL HISTORY:

INVESTIGATIONS:

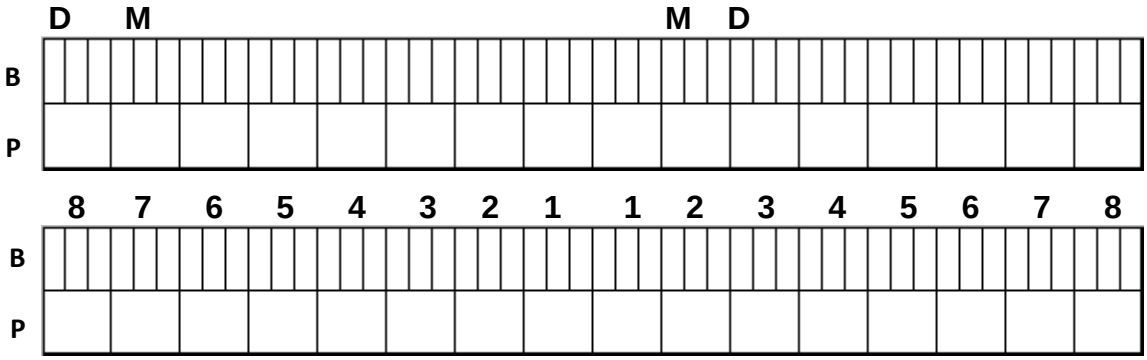
Blood-

IOPA-

CLINICAL EXAMINATION

PLAQUE INDEX

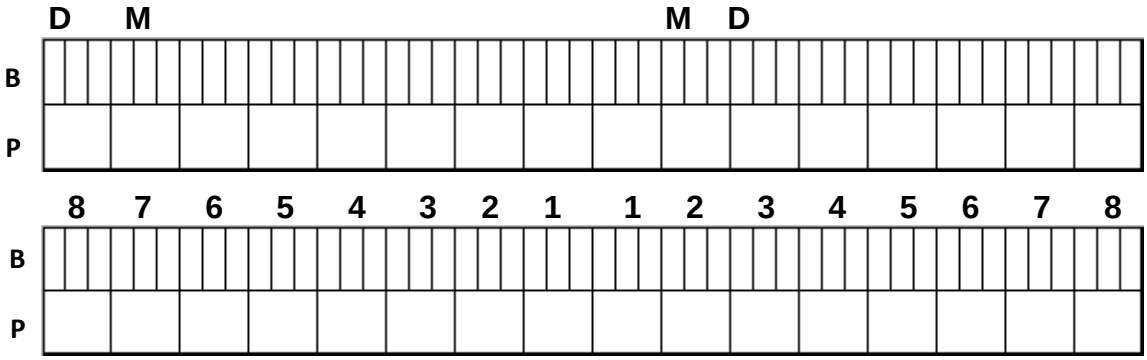
BASELINE:



SCORE ☐

GINGIVAL INDEX

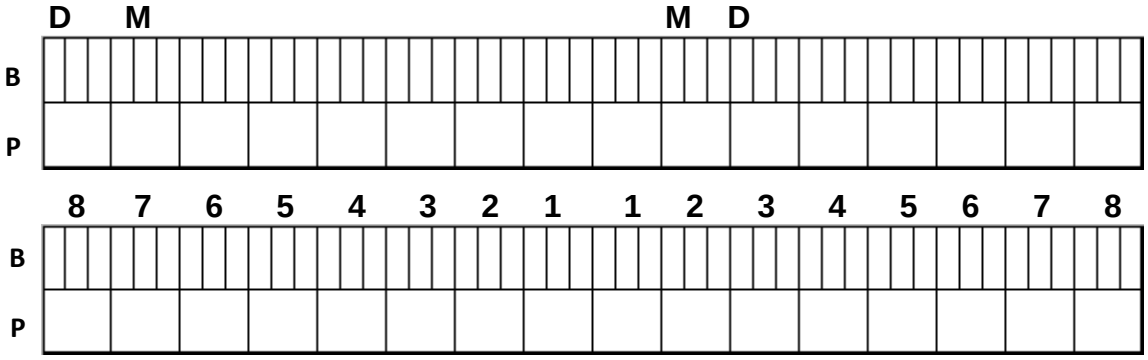
BASELINE:



SCORE ☐

SULCUS BLEEDING INDEX

BASELINE:



SCORE

Probing Pocket dept (mm)

No	Group I	Group II
PPD		

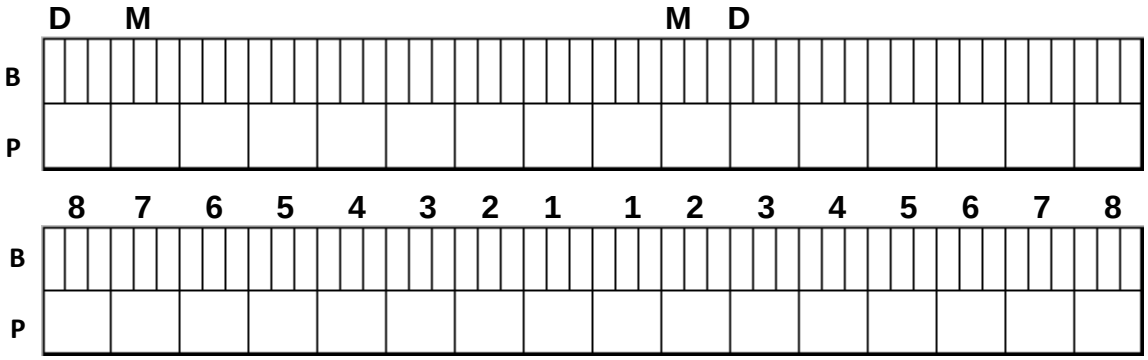
Biochemical Examination

	Group I	Group II
Superoxide dismutase level		

CLINICAL EXAMINATION

PLAQUE INDEX

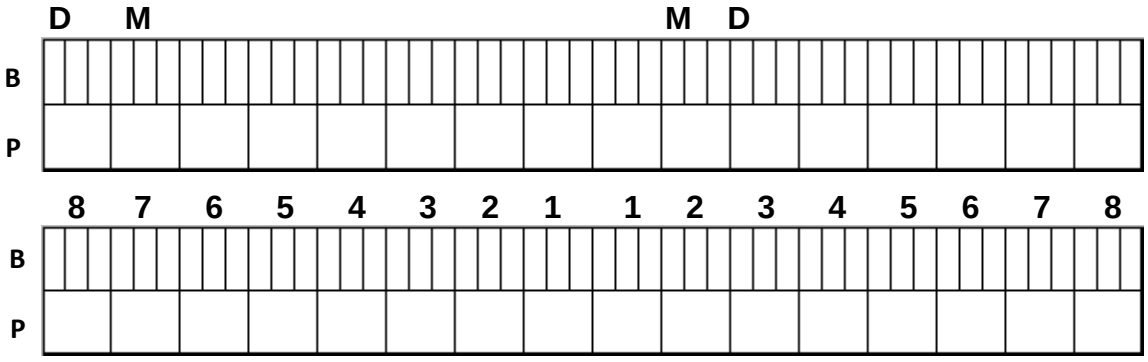
AFTER 21 DAYS:



SCORE ☐

GINGIVAL INDEX

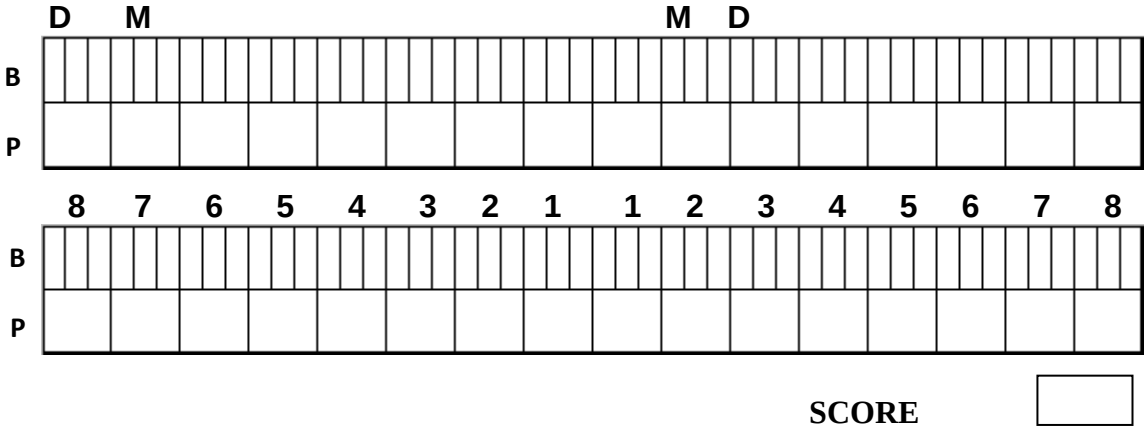
AFTER 21 DAYS:



SCORE ☐

SULCUS BLEEDING INDEX

AFTER 21 DAYS:



Probing Pocket dept (mm)

No	Group I	Group II
PPD		

Biochemical Examination

	Group I	Group II
Superoxide dismutase level		

INFORMED CONSENT OBTAINED FROM THE PATIENT

Department of Periodontics, J.K.K. Nattraja Dental College, Komarapalayam, Namakkal District, Tamilnadu.

Patient name:

I have been explained about the nature and purpose of the study in which, I have been asked to participate. I understand that I am free to withdraw my consent and discontinue at any time without prejudice to me or effect on my treatment.

I have been given the opportunity to question about the material and study. I have also given the consent for photographs to be taken at the beginning, during and end of the study.

I hereby give the consent to be included in "EVALUATION OF EFFICACY OF 0.2% CURCUMIN (CURCUMA LONGA) STRIP AS LOCAL DRUG DELIVERY SYSTEM AS AN ADJUNCT TO SCALING AND ROOT PLANING IN CHRONIC PERIODONTITIS- A CLINICAL AND BIOCHEMICAL STUDY".

Station: **SIGNATURE OF PATIENT**

Date: **SIGNATURE OF PROFESSOR**

APPENDIX - 2

ARMAMENTARIUM

MATERIALS AND INSTRUMENTS USED FOR THE STUDY:

- Gloves
- Mouth mask
- Patient apron
- Chair apron
- Head cap
- Sterile cotton rolls
- Gauze
- Saline
- Kidney tray
- Betadine
- Lignocaine
- Syringe
- Mouth mirror
- Straight probe
- Explorer
- Williams periodontal probe
- Tweezer

- Tissue holding forceps
- Hu-Friedy Gracey Curettes
- Microcapillary pipettes
- Eppendorf tubes
- 0.2% Curcumin strip
- Scissors
- Coe pak.

0.2% LOADED CURCUMIN STRIP

ARMAMENTARIUM

CLINICAL CASES

Pre operative

GCF COLLECTION

CONTROL GROUP

TEST GROUP

CLINICAL CASES

Curcumin Strip Placement

CURCUMIN STRIP PLACED

COE PAK PLACED

LAB ANALYSIS

RESULTS OF ELISA

This study consists of 20 subjects divided into 3 groups i.e., healthy group , chronic periodontitis (Control group – SRP alone) and (Test group – SRP + Curcumin strip), aged between 35-55 years from whom GCF was collected to estimate the concentration of Superoxide dismutase using ELISA and expressed as ng/ml.

STATISTICAL ANALYSIS

The results obtained were analyzed statistically and comparisons were made within each group using paired and unpaired student t test using SPSS 19.0 version software. A value of $P<0.05$ was considered as the level of significance.

CLINICAL EVALUATION:

No adverse reaction was observed in any subject, and no patient reported any discomfort. Healing was uneventful. All subjects tolerated the drug very well and without any post operative complications.

Plaque Index (PI):

The plaque index (PI) scores at baseline was 1.862 ± 0.539 and after 21 days PI scores reduced to 1.170 ± 0.192. Comparison of the PI scores between baseline and after 21 days shows a statistically significant difference with a P-value of <0.05.

Gingival Index (GI):

The gingival index (GI) scores at the time of baseline was 1.885 ± 0.595 and after 21 days GI scores reduced to 1.160 ± 0.239. Comparison of the GI scores

between baseline and after 21 days shows a statistically significant difference with a P-value of <0.05.

Sulcus Bleeding Index (SBI):

The sulcus bleeding index (SBI) scores at baseline was 2.027 ± 0.444 and after 21 days SBI scores reduced to 1.186 ± 0.212. Comparison of the SBI scores between baseline and after 21 days shows a statistically significant difference with a P-value of <0.05.

Probing Pocket Depth (PPD):

The probing pocket depth (PPD) in group I at baseline was 5.0 ± 0.0 mm and the PPD after 21 days reduced to 2.467 ± 0.833 mm. Comparison of the PPD scores in group I at baseline and after 21 days shows a statistically significant difference with a P-value of <0.05.

The probing pocket depth (PPD) in group II at baseline was 5.0 ± 0.0 mm and the PPD after 21 days reduced to 2.400 ± 0.828 mm. Comparison of the PPD in group II at baseline and after 21 days shows a statistically significant difference with a P-value of <0.05.

The probing pocket depth (PPD) in group I and group II after 21 days was 2.467 ± 0.833 mm and 2.400 ± 0.828 mm respectively. Comparison of the PPD in group I and II shows that statistically there is no significant difference with a P-value of >0.05.

Superoxide Dismutase Levels (SOD):

In healthy group, the mean concentration of superoxide dismutase levels were 12.264 ± 2.130.

In control group, at baseline the superoxide dismutase levels were 1.448 ± 0.625 and 21 days after treatment SOD levels improved to 9.366 ± 2.609. Comparison of SOD levels at baseline and after treatment shows a statistically significant difference with a P-value of <0.05.

In test group, the superoxide dismutase levels at baseline was 1.618 ± 0.746 and 21 days after treatment SOD levels improved to 11.649 ± 2.884. Comparison of SOD levels at baseline and after treatment shows that there is a statistically significant difference with a P-value of <0.05.

In control and test groups, the SOD levels after treatment was 9.366 ± 2.609 and 11.649 ± 2.884. Comparison of SOD levels post operatively in both groups showed that there is a statistically significant difference with a P-value of <0.05.

TABLE 1

Comparison of clinical parameters at baseline and after 21 days

Clinical parameters	At baseline	21 days	P value
Plaque index	**1.862 ± 0.539**	**1.170 ± 0.192**	**< 0.05**
Gingival index	**1.885 ± 0.595**	**1.160 ± 0.239**	**<0.05**
Sulcus bleeding index	**2.027 ± 0.444**	**1.186 ± 0.212**	**<0.05**

p- value between baseline and after 21 days is<0.05 denotes statistically significant at 5%

TABLE 2

Comparison of probing pocket depth between group I and group II

Clinical parameters	Group I	Group II	P value
Probing pocket depth	**2.467 ± 0.833**	**2.400 ± 0.828**	**>0.05**

p- value between group I and II is >0.05 denotes statistically insignificant at 5%

TABLE 3

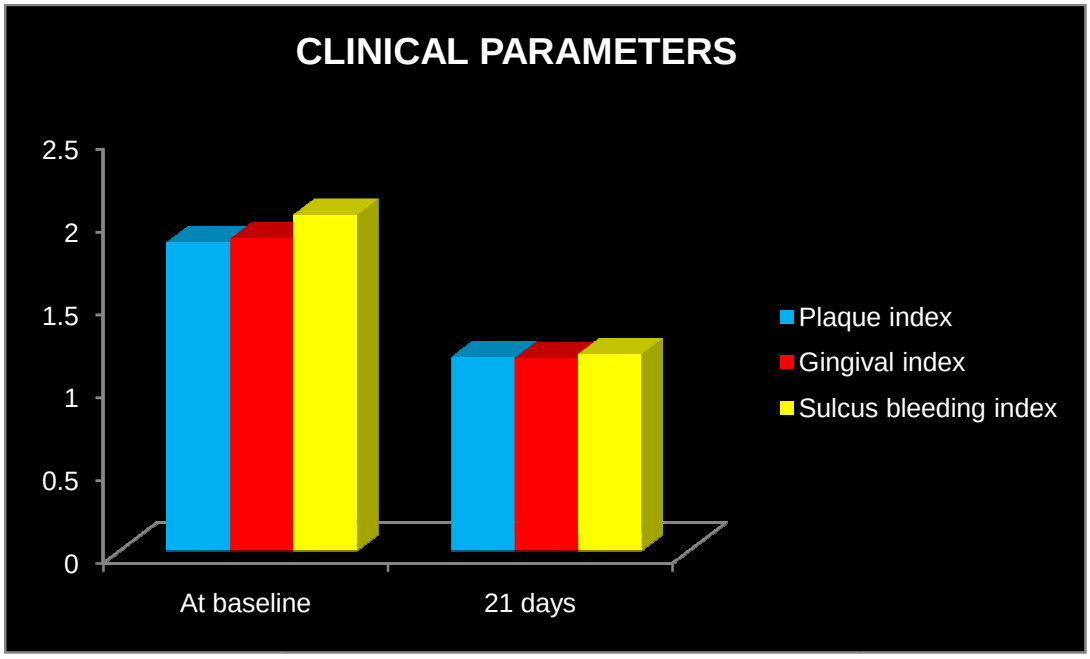
Comparison of SOD levels between groups

LEVELS OF SOD	Mean ± SD	P value
HEALTHY GROUP	**12.264 ± 2.130**	
GROUP I	**9.366 ± 2.609**	**<0.05**
GROUP II	**11.649 ± 2.884**	**<0.05**

p value between group I and group II is <0.05 denotes statistically significant at 5%

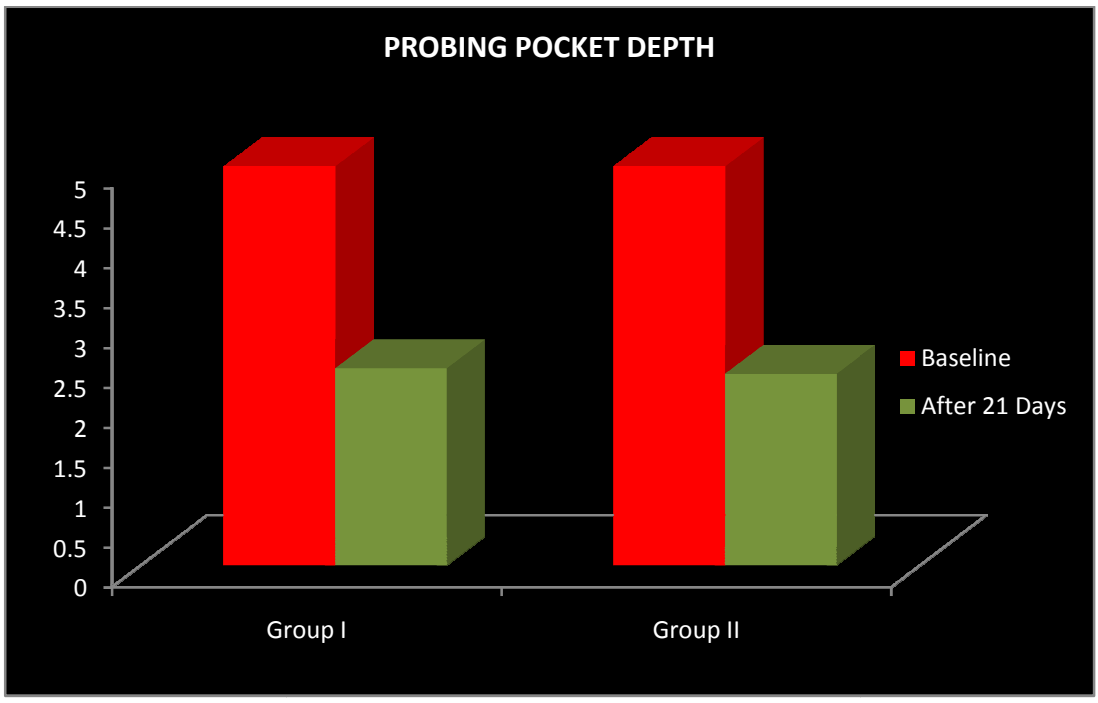
GRAPH - 2

Comparison of mean changes in Clinical Parameters at baseline and 21 days



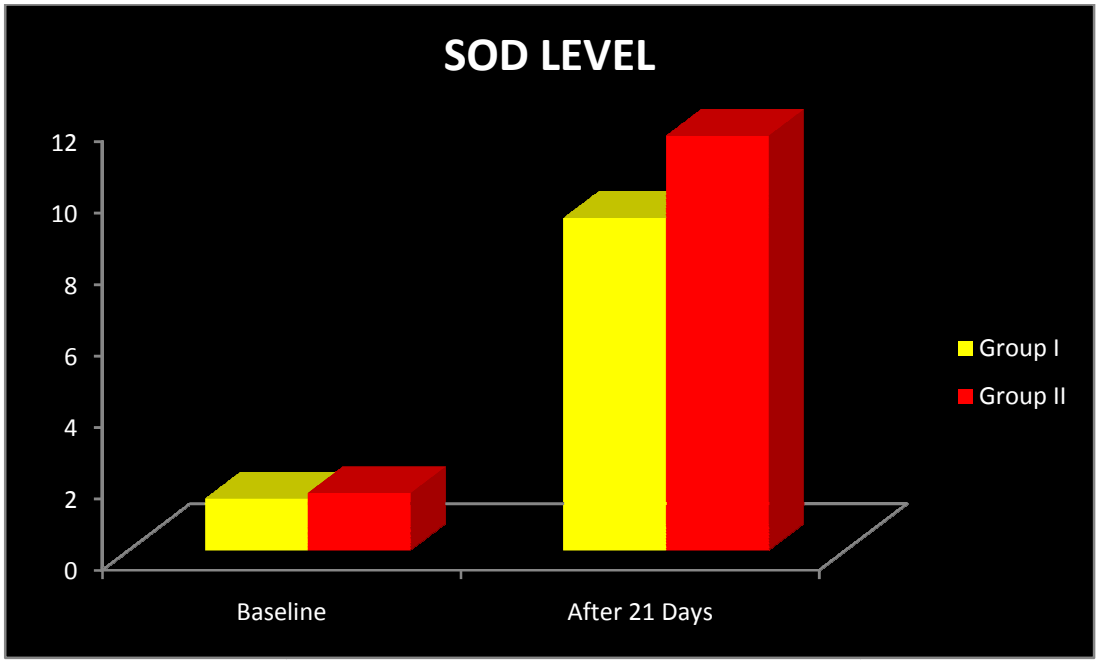
GRAPH – 3

Inter group comparison of mean changes of Probing Pocket Depth in Group I and Group II



GRAPH - 4

Inter group comparison of Superoxide dismutase levels



Periodontal disease is a multi factorial disease caused by microbial flora which is present in the subgingival plaque. Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans are the main periodontal pathogens responsible for the periodontal destruction.[2] Chronic periodontitis is more prevalent in periodontal sites exposed to these pathogens.

Traditionally periodontal therapy has involved in the reduction or elimination of these pathogens.[3] Nonsurgical periodontal therapy may not completely eradicate these periodontal pathogens, which leads into adjunct systemic antibiotic therapy for complete elimination of the pathogens.[4]

Systemic antibiotic therapy has the action of eliminating all periodontal pathogens but few shortcomings are present. Some disadvantages such as inability of systemic drugs to achieve high GCF concentration, increased risk of adverse drug reactions, increased multiple antibiotic resistant microorganisms and uncertain patient compliance.[5] These shortcomings led the invention of other possibilities as local drug delivery system.

Local drug delivery systems as local irrigation may not be more effective in periodontal disease management due to inadequate drug penetration into the deep pockets.[34] In sustained release system, higher drug concentrations are achieved at the disease site which reduces side and toxic effects. This system is considered to have excellent potential as an adjunct to traditional periodontal therapy.[33]

Tetracycline fibers[8], chlorhexidine chips[13] are the few successfully used sustained release system in periodontal therapy. Their sustained release, increases

many fold GCF drug concentration than systemic therapy. Chemical agents used in these systems are relatively expensive.

Instead of chemical agents, natural products could be used in the sustained release systems. With the growing interest and increasing knowledge about medicinal value of natural products, various formulations like Eucalyptus extract[57], blood root[58], chamomile[60], green tea catechin[62], aloe vera[64,] tulsi[65], and turmeric[15](Curcuma longa) are available in the drug delivery systems.

Curcumin is a yellow pigment from Curcuma longa, is a major component of turmeric and is commonly used as a spice and food-coloring agent. [76]

The desirable preventive or putative therapeutic properties of curcumin have also been considered to be associated with its antioxidant, anti-inflammatory, antimicrobial and chemopreventive properties. The anti-inflammatory effect of curcumin is most likely mediated through its ability to inhibit cyclooxygenase-2 (COX-2), lipoxygenase (LOX), and inducible nitric oxide synthase (iNOS). COX-2, LOX, and iNOS are important enzymes that mediate inflammatory processes. Curcumin works to inhibit the activity and synthesis of the enzymes implicated in inflammation, such as, COX-2 and 5-LOX.[74]

Curcumin may function indirectly as an antioxidant by inhibiting the activity of inflammatory enzymes or by enhancing the synthesis of glutathione, an important intracellular antioxidant. Curcumin acts as a free radical scavenger and antioxidant, inhibiting lipid peroxidation and oxidative DNA damage.[77]

Antioxidants are groups of substances that are able to prevent the oxidation of substrate by these ROS, thereby offering protection. Currently, there is a growing

interest in the linkage between antioxidants and periodontal disease. A significant antioxidant enzyme within mammalian tissues is superoxide dismutase, which catalyzes the dismutation of O_2^- to H_2O_2 and O_2. Superoxide dismutase has also been localized within the human periodontal ligament and may represent an important defense mechanism within gingival cells against superoxide release. treatment of periodontal disease reduces oxidative stress by a concomitant reduction in inflammatory load by enhancing antioxidant levels, irrespective of the medium, GCF or saliva.[18] Hence, curcumin has possessing the antioxidant activity and is used in this present study.

Efficacy of 0.2% curcumin is loaded onto the GTR membrane adjunct to SRP was evaluated in the present study. Strip form was used in this study for sustained release of drug for around 24 hours. According to Franz diffusion method[78], maximum release of drug from strip was within 24 hours and used in this study.

The clinical parameters such as plaque index, gingival index, sulcus bleeding index were recorded at baseline and 21st day. Parameters like probing pocket depth were also recorded at baseline and 21st day.

Plaque index was significantly reduced at 21st day at test and control site. Plaque index score was 1.862 ± 0.539, while in 21st day was 1.170 ± 0.192 showed significant changes due to oral hygiene maintenance therapy. These results concur with the study conducted by Shermin Karim et al. 2012.[18]

Gingival index score 1.885 ± 0.595 at baseline, significantly reduced to 1.160 ± 0.239 at 21st day. Significant changes were observed between test and

control site. The study conducted by Adriana et al. 2001[15] who showed similar reduction of gingival index scores after periodontal therapy. According to Waerhaug in 1955[79], the complete removal of subgingival calculus under favourable conditions, lead to reformation of a normal epithelial cuff in areas earlier covered with calculus and results in more or less complete disappearance of inflammation.

In the present study at baseline, Sulcus bleeding index score was found to be 2.027 ± 0.444. This score reduced to 1.186 ± 0.212 at 21st day. This changes obtained in SBI scores at test and control site were statistically significant from baseline to 21 days. This is in accordance with the study conducted by Adriana et al. 2001.[15]

The mean probing pocket depth(PPD) was significantly reduced in the 21st day compared to the baseline in control sites. Mean PPD was 5.0 ± 0.0 mm at baseline and reduced to 2.467 ± 0.833 at 21st day in control sites. While the Mean PPD was 5.0 ± at baseline while 2.400 ± 0. 828 at 21st day in test sites showed that there is no significant difference between the control and test sites. The results were in accordance with the study conducted by Roobal Behal et al. 2011[15] who demonstrated PPD reduction following placement of 2% turmeric gel, when compared to SRP alone. According to Caton et al. 1980[79], periodontal pockets were treated with periodic root planing and soft tissue curettage, which resulted in the reformation of an epithelial lining.[78]

Gingival crevicular fluid samples were taken at baseline and after SRP (21st day). Superoxide dismutase was present in greater quantities in the GCF, with elevated levels in mild, moderate, and severe periodontitis. Hence the present study

was planned to evaluate the superoxide dismutase levels in GCF samples by Enzyme Linked Immunosorbent Assay (ELISA).[18]

According to Shermin Karim et al. 2012[18], superoxide dismutase presented higher levels in the GCF before treatment and improved following periodontal treatment. To detect the SOD levels enzyme linked immunosorbent assay performed in this study.

In healthy group, superoxide dismutase levels were 12.264 ± 2.130.

In the present study, at baseline SOD levels for control group was found to be 1.448 ± 0.625 and improved to 9.366 ± 2.609 at 21st day following periodontal therapy, which shows statistically significant differences before and after treatment.

Superoxide dismutase levels at baseline in test group was 1.618 ± 0.746 and improved to 11.649 ± 2.884 at 21st day following periodontal therapy, which shows statistically significant differences at baseline and following therapy. This shows that statistically there is a significant differences between the SOD levels following therapy when compared with the control and test groups.

The present study involved a comparative clinical and biochemical evaluation of 0.2% curcumin chip and SRP alone in the treatment of chronic periodontitis. The study population comprised of 5 healthy subjects and 15 subjects each with 5mm bilateral probing pocket depth were randomly divided into test and control groups. 0.2% curcumin strip was inserted in the test site, while SRP alone in control site. GCF samples were collected at baseline and 21st day to evaluate superoxide dismutase levels. Clinical parameters like Plaque index (PI), Gingival index (GI), Sulcus bleeding index (SBI), Probing pocket depth (PPD) were assessed at baseline and 21st day.

From this study, the following conclusions have been elucidated,

1. Sustained release systems prevent the recolonization of pathogens for long period and reduces inflammation.

2. Clinical parameters were significantly reduced in test and control sites.

3. Superoxide dismutase levels were significantly improved in test sites when compared with control sites.

Thus the present study concluded that, curcumin strip effectively used as an adjunct to scaling and root planing in treatment of chronic periodontitis and serves as antioxidant in subgingival environment.

Though, the study has positive outcome for particular antioxidant, elaborate studies are needed to prove the efficacy of curcumin strip in other antioxidants and also with reference to areas like proper concentration and release system.

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